Intense Human Impact on Microbial Contamination during the Ohio State University Annual Mirror Lake Jump 2010 and its Epidemiological Implications

Honors Research Thesis

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ABSTRACT

In order to assess the potential fecal contamination, and possible human health risk posed by an urban lake following a high human disturbance event on the Ohio State University campus, we examined human source contribution by measuring water quality and qPCR targeting multiple genetic markers (*tetQ, huBac gyrB,* and *Ent 23S*) indicative of the presence of fecal bacteria. During the night of November 23-24, 2010, hourly water samples were collected. Water quality, fecal indicator bacteria, and human-specific fecal bacteria were enumerated using standard methods. Amongst the parameters, *tet*Q (antibiotic-resistance gene, qPCR), human-specific bacteroides (qPCR), *E.coli* (culture method), and *Enterococci* spp. (culture method) increased throughout the night. This could be attributed to human-originated fecal contribution via bather's shedding because the observed culturable *Enterococci* spp. results are comparable to past studies showing a correlation between bather densities and *Enterococci* counts. Among the water quality parameters measured, turbidity showed significant changes. Number of *E. coli* and *Enterococci* peaked around the time of the highest human numbers, but decreased sharply afterwards. This can be attributed to the increased turbulence and the disturbed sediments due to the large number of students present in the water. However, the sharp decrease indicates that these bacteria attached to the disturbed soil particles which settled after the majority of the people had left. Therefore, turbidity must be considered when determining the contribution of sediments to the overall bacterial load. By determining whether an association exists between bather densities, observed bacterial counts, and qPCR results, this could lead to the future development of using qPCR of fecal bacteria for routine water quality monitoring of recreational bodies of water.

INTRODUCTION

In assessing water quality of recreational waters, advisories must be posted when the water quality exceeds US Environmental Protection Agency microbial standards for enterococci or *E. coli* concentrations in order to minimize public health risks (U.S. EPA, 1986). According to the latest Centers for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Report on recreational waters, the recreational swimming season of 2007-2008 marked the largest number of recreational water-associated illnesses since 2004, causing 134 outbreaks resulting in at least 13,994 cases (CDC MMWR 2007). Besides the public health risk posed by exceeded microbial standards, public beaches generate a significant amount of tourism and source of revenue in the U.S and the amount of money lost after beach closings due to public health advisories has been substantial in past years (NRDC, 2007). However, for the majority of the advisories posted the source of pollution has rarely been identified. This inability to identify the point sources of pollution hinders the development of effective strategies to minimize the contamination as well as decrease the number of annual beach closings.

Studies in the past have identified human bather shedding as a potential significant non-point source of fecal indicator bacteria, thus affecting the overall microbial water quality and the instances of swimming related illnesses. Calderon et al. (1991) found that as the number of bathers increased, the higher the incidence of gastrointestinal illness. Three other studies conducted by Robinton and Mood (1966), Hanes and Fossa (1970), and Smith and Dufour (1993) also deduced that swimmers contributed significantly to increasing bacterial densities in the water column. All of these studies were conducted in freshwater environments and were in relation to single bather events.

Enterococci have historically been recommended by the EPA as a reliable indicator of potential fecal contamination (US EPA, 1983, 1984, 2002). *Enterococci* are commonly found in human

feces as well as other warm-blooded species. Additionally, other recent studies conducted at the freshwater beaches of Lake Michigan (Whitman and Nevers, 2003) and Lake Huron (Alm et al., 2006) indicates that environmental media such as sand could serve as a source of these indicator bacteria. However, past studies have implied that *Enterococci* have been found to be naturally shed from bathers, rather than environmental sources (soil, sand, etc.) at significant concentrations. *Elmir* et al. (2007) found that by measuring *Enterococci* concentrations present after sequential bathing events, bathers shed significant amounts of *Enterococci* ($5.5x10^5$ CFU/bather), particularly after the first wash cycle was completed. Bather shedding was found to be the major contributor to the overall microbial contamination load rather than the beach sands or sediments (24 - 390 CFU/g-dry sand, representing less than 5% of the overall *Enterococci* total) that were also analyzed as a non-point source contribution.

In addition to using *Enterococci* as a fecal indicator organism, *E. coli* has also traditionally be used by the EPA to assess water quality (U.S. EPA, 1986). The latest health survey-based study correlating the incidence of waterborne diseases in recreational waters confirmed that the traditional EPA standard of measuring *E. coli* concentrations was a reliable indicator of the potential gastrointestinal illness risk posed at inland U.S. beaches (Marion *et al.*, 2010). *E. coli* have also traditionally been regarded as an indicator organism of fecal contamination. However, because of its presence in environmental mediums (i.e. soil), Fujioka et al. (1999) concluded that tropical environmental conditions supported the growth of *E. coli* in soils, so *E. coli* is not a reliable fecal indicator. Thus, the measurement of water turbidity, or the re-suspension of soil particulates, has long been established as a reliable proxy of the presence of enteric bacteria in aquatic sediments, such as those residing in lakes environments (Geldreich, 1972; Gerba and McLeod, 1976; LaLiberte and Grimes, 1982). Soil particles are sources of organic nutrients, thus encouraging bacteria to

attach to soil particles to prolong their survival (Gerba and McLeod, 1976). Sediments decrease competition between *E. coli* and other microflora in the water column, leading to the greater presence of *E. coli* (Gerba and McLeod, 1976). Soil particulates have also been shown to protect attached bacteria from UV radiation (Bitton et al., 1972), high salinity (Ghoul et al., 1986), toxicity from heavy metals (Jones, 1964), grazing by protozoa (Enzinger and Cooper 1976), and attack by bacteriophages (Roper and Marshall, 1979). In addition to enteric bacteria, sediments are capable of harboring viruses (LaBelle and Gerba, 1979, 1980; Xu et al. 1982).

Although the public health risk that high turbidity levels pose varies depending on the aquatic system, positive correlations between the occurrence of fecal coliforms, salmonellae, and viruses in sediments have supported increased monitoring of water usage and sediment screening (LaLiberte and Grimes, 1982). Additionally, previous studies have shown a correlation between raw water quality parameters such as turbidity, total, and fecal coliform measurements and the occurrence of waterborne illness protozoa such as *Giardia* and *Cryptosporidium* (LeChevallier et al., 1991). As a result, the disturbance of these sediments at Mirror Lake will lead to the release of these bacteria into the water column. This phenomenon was previously observed in the once highly-used, multi-purpose Buffalo Lake southwest of Amarillo, Texas that underwent almost continual disturbance (Geldreich, 1972). It was also observed following dredging in the Mississippi River navigation channel (Grimes, 1975). Thus, measuring the turbidity levels in these different aquatic environments is important in terms of the assessing the public health risks that re-suspended sediments pose for recreational or drinking water usage (Grimes, 1975; LaLiberte and Grimes, 1982).

In the past, culture-based methods, including membrane filtration (MF), have been employed as an efficient way to enumerate fecal bacteria that reside in environmental mediums such as soil

and water. However, this method does not allow for discrimination between animal and human-originated bacteria. Although the detection of general and human-specific Bacteroidales have not been employed in U.S. EPA routine water quality monitoring, previous studies have suggested that this approach could also be employed in more accurately assessing the human-specific fecal contribution to a body of water (Gawler et al., 2007; Walters et al., 2007, U.S. EPA, 2007). Thus, in 2009, Elmir et al. expanded their study to include chromogenic substrate IDEXX Enterolert™ (CS) to enumerate *Enterococci*, quantitative polymerase chain reaction (Haugland et al. 2005) to also enumerated *Enterococci*, and shedding of alternative fecal indicator bacteria Bacteroidales human markers UCD (Kildare et al. 2007) and HF8 (Bernhard and Field, 2000) by qPCR. It was found that the *Enterococci* levels were comparable to the 2007 study and qPCR results were expected to by similar or higher relative to the statistically similar MF and CS results. Additionally, the Bac-Hum UCD marker was found to be common amongst human populations, indicating that it could be useful in tracking future fecal contributions from people (Elmir et al., 2009). Besides being used in assessment of water quality of swimming pools, Okabe et al. (2007) suggested that using qPCR to enumerate *Bacteroides spp.*, in particular the *Bacteroides-Prevotella* 16S rRNA genetic marker, could serve as a reliable, organism-specific marker in assays monitoring fecal contaminations in natural water environments. Nikolich et al. (1994) discovered that the tetracycline resistance gene (*tetQ*), formally prevalent in farm animal lumen, had been genetically horizontally transferred and was now present in several human-specific *Bacteroides* species and could thus be used as a human fecal contamination indicator. Recently, C.S. Lee and J. Lee (2010) utilized an alternative *Bacteroides* housekeeping gene, *gyrB*, as a qPCR assay target. Results indicated that the high host-specificity of *gyrB* was promising in rapidly detecting and identifying human-specific fecal contamination. Overall, utilizing qPCR methods to detect the presence of *Bacteroides* has led to a

more accurate assessment of human fecal impact on water quality. Additionally, human enteric viruses have been utilized in qPCR due to their strong association of causing gastroenteritis cases worldwide (Kohli et al. 1999; Vinje et al. 1997). Human adenovirus, human enterovirus, and norovirus have been shown to be detectable in freshwater environments (Haramoto et al. 2005), as well as be quantified by qPCR (Monpoeho et al., 2000; Katayama et al., 2002; Kageyama et al., 2003; Heim et al., 2003).

In this study, a comprehensive assessment of water quality, chemical, and biological parameters were measured on Mirror Lake, a small man-made lake on the Ohio State University campus, Columbus, Ohio. The night before the annual football game between Ohio State and the University of Michigan, students traditionally participate in the "Mirror Lake Jump," in which hundreds of students jump into the lake on campus. The risks to students' health posed by this event have never been assessed until now. In addition to being the first study to uncover the ecological impact on a body of water after an intense human-impact event, this is the also the first health risk assessment done on a single event with such a large number of people involved in lake containing a known volume of water. As well as measuring traditional water quality parameters, we also determined a broad spectrum of microbes, including *E. coli, Enterococcus* spp., *Bacteroides* spp., and human adenovirus, human enterovirus, and norovirus in the water samples in order to assess the microbial water quality deterioration during the duration of the event and resulting public health risk. Changes in *Bacteroides* and *Enterococcus* spp. may provide a direct link between human bather shedding of fecal bacteria and decreasing water quality. By employing culture-based methods to enumerate traditional fecal indicator organisms, and the quantitative PCR to enumerate human-specific fecal bacteria gene markers, this will provide insight into more thorough quantitative understanding of human-originated fecal contamination.

The conclusions can serve as a predictor for the overall human health risk that is posed by bodies of water after extreme disturbance events involving massive bather shedding and turbidity.

MATERIALS AND METHODS

SAMPLING SITE

All of the samples were gathered from Mirror Lake, a small urban lake containing 91,000 cubic feet of water (OSU University Engineer's Office) located in the middle of the Ohio State University campus. The lake is surrounded by natural foliage and is home to a few mallard ducks and Canada geese. In total, eight water samples were gathered hourly between 6 PM on November 23, 2011 and 2 AM on November 24, 2011. Sampling began at 6 PM before any swimmers had entered the pond as a control sample for the experiment. During each sampling time, triplicate one-liter surface water samples were gathered from the southern bank of Mirror Lake using 1 L Nalgene bottles (Figure 1). An additional 500 mL of water were gathered in Whirlpak© bags for phosphorus concentration tests. Figure 1 details the Mirror Lake layout as well as the location of the sampling site. This single sampling location was chosen because of its close proximity to a highest number of jumping participants. Additionally, this location would most accurately represent the water composition to which swimmers would be exposed during the event, thus aiding in epidemiological assessment of this event.

WATER QUALITY MEASUREMENTS AND ENUMERATION OF PARTICIPANTS

During each sampling period, water quality measurements were taken on-site using a YSI© 650 MDS Probe (YSI Inc., Yellow Spring, OH), including temperature (°C), pH, specific conductivity (S/cm), and dissolved oxygen (mg/L) per the manufacturer's instructions. While measurements were taken, the number of participants at each time was estimated by eye and by photographing the entire lake.

CHEMICAL PARAMETER MEASUREMENTS

All water samples were stored on ice and then immediately transported to the lab for testing. The chemical parameters measured included turbidity, total chlorine, total ammonia, total phosphorus, phycocyanin, and chlorophyll a. Turbidity, total chlorine, total ammonia, and total phosphorus are typically measured to assess water quality. Both chlorophyll a (Caraco and Puccoon, 1986) and phycocyanin (photosynthetic accessory pigment) (Bogorad 1975) are found in algal matter and photosynthetic organisms. Turbidity was measured using HACH© Method 8366 (Determining the Relationship between Turbidity and Total Suspended Solids). Total chlorine was measured using HACH© US EPA DPD Method 8167 (0.02-2.00 mg/L). Total ammonia was measured using HACH© US EPA Nessler Method 8038 (0.02-2.5 mg/L NH_3-N). Total phosphorus was measured using HACH© US EPA PhosVer® 3 with Acid Persulfate Digestion Method 8190 (Test 'N Tube™ Vials - 0.06-3.5 mg/L PO_4^{3-} or 0.02-1.10 mg/L P). Phycocyanin and chlorophyll *a in-vivo* were measured using the dual-channeled *Aqua*Fluor® Handheld Fluorometer/Turbidimeter (Turner Designs® - Sunnyvale, CA). All chemical measurements were compared against previously developed standard curves.

E. COLI AND *ENTEROCOCCI* ENUMERATION

All samples were stored on ice and transported immediately to the lab for testing. Samples were then filtered using Millipore© 20 μm (pre-filter) and 0.45 μm cellulose filters with three different volumes of water (20, 50, and 100 mL) for bacterial enumeration. These filters were then plated on mTEC and mEI agar to enumerate *E. coli* and *Enterococci* according to standard U.S. EPA Procedure Methods (Method 1603, U.S. EPA 2002; Method 1600, U.S. EPA 2002). All *E. coli* m-TEC plates were first incubated at 35 ± 0.5°C for 2 hours before being transferred for 44.5 ± 0.2°C incubation overnight for 24 h. *Enterococci* m-EI plates were incubated at 41.5 ± 0.5°C for 24

h. Purple *E. coli* and blue *Enterococci* colonies were then enumerated.

HUMAN-SPECIFIC FECAL ORGANISM ENUMERATION VIA Q-PCR

500 mL of each water sample were filtered through a Millipore© 0.45 µm cellulose filter and the bacterial DNA was extracted from the filter by using a Qiagen© QIAamp DNA Mini Kit according to the manufacturer's procedure. Using the DNA, genetic markers of human-specific fecal organisms were enumerated via q-PCR assays (TaqMan® and SYBR®-Green). In brief, for the Taqman® qPCR assays, 1 µl of the sample DNA, 0.1 µl each of the forward and reverse primers, 10 µl of two times TaqMan® Universal Master Mix, 0.05 µl of TaqMan® probe, and 8.8 µl of sterile PCR grade water (from master mix kit) were used giving a total volume of 20 µl. In brief, for the SYBR®-Green qPCR assays, 1 µl of the sample DNA, 0.1 µl each of the forward and reverse primers, 10 µl of SYBR®-Green PCR Master Mix, and 8.8 µl of sterile PCR grade water (from master mix kit) were used giving a total of 20 µl. All analyses were run twice and the averages of the duplicate analyses were reported. *Enterococci faecalis* cultures and *Bacteroides fragilis* cultures from the American Type Culture Collection (ATCC) were used as internal genomic control standards. Primers were used to quantify *Bacteroides/Prevotella* 16S rRNA gene (huBac) (TaqMan® - qHS601F/qBac725R/qHS624MGB) (Matsuki et. al 2002; Okabe et al. 2007), *B. fragilis gyr*B gene (*gyrB*) (TaqMan® - Bf904F/Bf958R/bf923MGB)(C.S. Lee and J. Lee, 2010), *tetQ* gene (*tetQ*) (SYBR®-Green – tetQF/tetQR) (Nikolich et al., 1994), and *Enterococci* 16S rRNA gene (Ent 16S) (TaqMan® - ECST748F/ENC854R/GPL813TQ) (Haugland et al., 2005).

ENUMERATION OF HUMAN NOROVIRUS, HUMAN ADENOVIRUS, AND HUMAN ENTEROVIRUS VIA REVERSE TRANSCRIPTION PCR AND QPCR

The DNA and RNA of Human norovirus, human adenovirus, and human enterovirus were extracted and concentrated from frozen water filters via previously established methods (Monpoeho

et al. 2000; Katayama et al. 2002; Haramoto et al. 2005). In brief, 500 ml of each water sample was filtered through an $AlCl_3$ cation-coated membrane and then cations were eluted via 0.5 mM H_2SO_4. The viruses were then eluted by the addition of 1 mM NaOH to the filter. Because human norovirus and human enterovirus are both RNA viruses, these were quantified via reverse-transcription PCR (Kageyama et al. 2003). In brief, 1 µl of the sample cDNA, 0.15 µl each of the forward and reverse primers, 5 µl of five times Q Buffer, 2 µl of five times Q Solution, 1 µl of dNTP mix, 0.075 µl of probe, 0.075 µl of ROX, 1 µl of enzyme mix, 0.2 µl of RNA Inhibitor (RNAse Out), and 14.4 µl of sterile RNAse-Free water (from master mix kit) were used giving a total volume of 20 µl. In brief, for the SYBR®-Green qPCR assays, 1 µl of the sample DNA, 0.1 µl each of the forward and reverse primers, 10 µl of SYBR®-Green PCR Master Mix, and 8.8 µl of sterile PCR grade water (from master mix kit) were used giving a total of 25 µl. Human adenovirus (DNA virus) was quantified via methods developed by Heim et al. (2003) using TaqMan® real-time PCR. In brief, 1 µl of the sample DNA, 0.1 µl each of the forward and reverse primers (AQ1/AQ2), 10 µl of two times TaqMan® Universal Master Mix, 0.05 µl of TaqMan® probe (AP), and 8.8 µl of sterile PCR grade water (from master mix kit) were used giving a total volume of 20 µl.

RESULTS

Biological parameters of *E. coli* (m-TEC) and *Enterococci* (m-EI) plate count enumerations, turbidity, and the number of participants are shown in Figure 2. Turbidity showed a strong correlation with the number of participants present in Mirror Lake at each sampling time. As the number of participants increased until midnight and then decreased from 1 AM to 2 AM, turbidity measurements also reflected this pattern. Additionally, the plate counts of *E.coli* and *Enterococci* correspondingly increased as turbidity increased. During the sampling period with the highest turbulence and largest number of people (12 AM, 28.7 NTU, 575 people), the peak *E. coli* and

Enterococci concentrations of the night were 2.72 log CFU/100 ml and 3.08 log CFU/100 ml respectively.

Of the water quality parameters measured, only turbidity showed a significant change throughout the night. Water quality parameters measured by the YSI probe and chemical concentration measurements are shown in TABLE 1. The other water quality parameters of temperature (°C), pH, conductivity (S/cm), and DO (mg/L) did not vary significantly.

At the highest turbulence time (12 AM), the total chlorine and total phosphorus concentrations peaked at 0.22 and 0.61 mg/L, respectively. In general, total chlorine and total phosphorus followed the same increase and then sharp decrease as turbidity measurements that also correlated with the number of participants at each sampling period. However, total ammonia gradually increased and then stayed slightly elevated in comparison to initial levels (0.18 mg/L at the peak turbulence time of 12 AM and 0.19 mg/L at 1 AM). Chlorophyll a is found in some algal matter and its increased concentrations at 12 AM, 1 AM, and 2 AM (56.79, 48.68, and 66.70 μg/L respectively) indicate that these photosynthetic organisms may have been re-suspended in the water column from the disturbed sediments.

The fecal genetic marker concentrations are shown in FIG. 3. Most increased in accordance with the number of swimmers present, indicating that bathers may have significantly contributed to the overall contamination of the water throughout the night. Among the genetic markers measured, *TetQ* and human *Bacteroides* (*huBac*) displayed constant upward trends in concentration throughout the night, peaking at 2 AM (5.01 CFU/100 ml, 3.69 CFU/100 ml, and 4.72 CFU/100 ml respectively). These trends could indicate that although the turbidity and particulate matter decreased as the number of participants decreased towards the end of the event, these human-specific fecal bacteria remained suspended in the water column for a long time.

Human nororovirus, human adenovirus, and human enterovirus were not detected.

DISCUSSION

The results for the water quality, chemical, and biological parameters that were measured in this experiment strongly indicate that there was a severe human impact on the microbial quality in the lake during the jump. The culturable levels of *E. coli* and *Enterococci*, combined with the elevated qPCR results for *TetQ*, huBac, and Ent 23S, are indicative of human fecal contamination, most likely attributed to bather shedding due to the large numbers of swimmers present during the event.

Although quantitative data assessing the enteric pathogen contribution from people in recreation waters is generally not available, Gerba (2000) conducted a literature assessment to estimate the relative posed bather fecal coliform contamination threat to drinking water reservoirs. In particular, the *Enterococci* counts in this study were comparable to the concentrations found in previous studies involving bather shedding of significant amounts of fecal indicator bacteria into the water column shown in TABLE 2. TABLE 2 outlines the calculations performed to provide a risk assessment of the approximate *Enterococci* concentrations that the swimmers may have been exposed to during the event. The expected *Enterococci* concentrations that swimmers may have realistically been exposed to between the hours of 6 PM – 11 PM, using the experimental models of Smith and Dufour (1993) and Elmir et al. (2007), fell within the expected ranges ($1.2x10^5$ – $5.5x10^5$ CFU shed/bather) described by these two studies, in particular between the hours of 10 PM – 12 AM. This finding implies that the high numbers of people that participated in the event contributed significantly to the fecal bacterial contamination observed in Mirror Lake. An anomaly to the calculations was our peak *Enterococci* concentration measured at 12 AM during which time the

highest number of swimmers was also observed. Our 12 AM *Enterococci* concentration of 9.4 x 10^3 CFU/100 ml was a magnitude of 10 times higher than expected, but several explanations are available. First, most of the participants during this hour were presumed to have been somewhat inebriated in celebration of this school spirit event, may have had less bodily control of their fluids during the event, and thus more accidental fecal releases were likely to occur. Gerba (2000) found that even on at a frequency of accidental fecal releases of one per 1000 people, this one accident would still have a significant impact on the concentration of fecal contamination in the water column. Given the total number of people that potentially participated in the event (~48,420 people), the likelihood of accidental fecal releases was likely quite high with an estimated number of 48 fecal accidents if not more. This is especially reflected by the calculations prior to 12 AM that were reasonable within the contexts of the other two studies, but yet the 12 AM concentration suddenly spiked when the dynamics of the crowd also changed. Second, the participants described in the previous two studies were completely sober and had been previously informed that they were being tested for bather shedding rates. Finally, this last concentration of 9.4 x 10^3 CFU/100 ml also reflects the contributions of the potential 13,920 participants that jumped into the lake prior to 12 AM. Thus, this count would be expected to be higher due to accumulation of fecal contribution from the previous hours.

Of particular note is that two human-specific fecal genetic markers, *TetQ* and huBac, rose in concentration throughout the night as the levels of *Enterococci* also increased. Regression analysis revealed a slight association when comparing *Enterococci* vs. huBac ($R^2 = 0.7411$) and *Enterococci* vs. *TetQ* ($R^2 = 0.4426$). Further replicates of sampling events need to be conducted in order to assess the strength of association between these genetic markers and *Enterococci*. However, this slight correlation between plate counts for *Enterococci* and qPCR results for the human-specific

fecal genetic markers (Haugland et al., 2005) match findings in previous studies that imply that *Enterococci* could be used for assessing microbial water quality in recreational bodies of water (Nikolich et al., 1994; Okabe et al., 2007).

Another significant finding was that *E. coli* and *Enterococci* concentrations strongly correlated with the observed levels of turbidity, particularly in regards to the rise and fall of both indicator organisms and the corresponding amount of turbidity. This correlation was statistically significant in regression analysis when comparing *E. coli* vs. *Enterococci* ($R^2 = 0.9287$), *E. coli* vs. Turbidity ($R^2 = 0.8934$), and *Enterococci* vs. Turbidity ($R^2 = 0.9096$). Additionally, the slightly elevated levels of chlorophyll a imply that photosynthetic organisms that normally would not be on the surface waters at night were re-suspended due to turbulence. Previous studies have indicated that *E. coli*, *Enterococci*, and other fecal bacteria may ubiquitously exist in several environmental mediums such as soil (Allen et al. 1953; Van Donsel and Geldreich 1971; LaLiberte and Grimes 1982; Doyle et al. 1992; Fujioka et al.,1999). In addition to soil contribution of these naturally-occurring environmental indicator organisms, the mallard ducks and Canada geese that live around the lake could have also contributed to the observed *E. coli* and *Enterococci* counts. Previous studies have shown that ducks shed *E. coli* and *Enterococci* bacteria in their feces (Geldreich et al. 1962; Murphy et al., 2005). Wright et al. (2009) conducted a beach study assessing microbial loads from different animals and suggested that ducks had an *Enterococci* contribution of approximately 1×10^4 CFU/ g of dry feces. Canada geese have been shown to shed these organisms as well (Middleton and Ambrose, 2005) with *E. coli* counts ranging from 0-1.0×10^7 CFU/0.1 g wet weight of feces and *Enterococci* spp. counts ranging from $1.0 \times 10^2 - 1.0 \times 10^7$ CFU/g wet weight of feces. However, these counts from waterfowl contribution are unlikely to be seen in Mirror Lake, mainly because these counts were enumerated from fresh feces and were not diluted by water or any other

environmental media. Additionally, given the time of year in which the experiment was conducted (November), the residual indicator bacteria that may have been naturally growing in the soils or were contributed by water fowl would not have a significant contribution to the overall fecal indicator organism counts that were observed because of the increased mortality rate of these bacteria in a cooler environment. (Van Donsel et al., 1967). Because of the significant *E.* coli and *Enterococci* counts that were observed, these can be more closely attributed to fresh human input rather than naturally occurring levels or waterfowl contributions.

The inability to detect viruses could be due to our filtered volume of water not containing a high enough concentration of viruses to reach the detection limit of the assay. This underrepresented virus enumeration was also shown in previous studies conducted on water samples that used the same volume of water analyzed in our experiment alone without sediment analysis (LaBelle and Gerba, 1979; 1980).

If students decide to participate in the Mirror Lake Jump event, it is advisable that they jump during the earlier part of the night before the higher numbers of swimmers arrive later and contribute significant amounts of bacteria from bather shedding. The turbidity of the water was a strong indicator of the level of fecal contamination during the event (LaLiberte and Grimes, 1982) in terms of predicting *E .coli* and *Enterococci* levels present. As stated previously, the sampling time with the highest turbulence and largest number of people (12 AM, 28.65 NTU, 575 people) also had the highest concentration of *E. coli* (2.72 log CFU/100 ml or 4590 CFU/100 ml) and *Enterococci* (3.08 log CFU/100 ml or 9400 CFU/100 ml). For a comparison to the health risk that this concentration of *E. coli* poses, according to the latest EPA regulations on recreational water quality criteria, if culturable *E. coli* levels exceed 235 CFU/100 ml, beach advisories must be posted to warn swimmers of the elevated health risks (U.S. EPA, 1986). The peak *E. coli* concentration

measured at 12 AM in this experiment was roughly twenty times higher than the EPA limit. Additionally, the EPA regulation on culturable *Enterococci* for posting beach advisories is 61 CFU/100 ml (U.S. EPA, 2000). In our experiment at the peak activity time, our *Enterococci* concentrations were roughly 154 times higher than this EPA limit. Thus, the public health risk posed by this event is quite significant. Especially of note is that the *Enterococci* counts observed are significantly higher than *E. coli* counts, a ratio not usually expected in environmental studies, as seen by the EPA regulation for posted beach advisories. This further strengthens the argument that human fecal matter contributed to these observed higher *Enterococci* counts rather than natural sources such as water or soil.

However, the new evidence of our observed *Enterococci* levels being comparable to levels found in bather shedding studies, as well as the correlation suggested by increased fecal genetic markers, indicates that *Enterococci* could be used as an indicator of recent human fecal contamination, thus providing a broader scope of the public health risk posed by this event. This event also gives insight into the significant fecal contribution during such a large single bathing event. If students choose to participate in the event, they should rinse themselves off immediately, especially around the face, nose, mouth, and hands to prevent contact and/or ingestion of possible pathogens.

Overall, the Mirror Lake Jump poses a significant health risk due to the significant levels of fecal bacteria contributed by bather shedding, as shown by the elevated presence of the indicator bacterium *Enterococci.* The health risk could increase exponentially if participants undergo extended exposure time in the water, especially if their faces, mouths, and eyes come into contact with the water. Additionally, bacteria could become airborne during periods of high turbulence (via splashing), and pose a health risk to bystanders.

Among the chemical water quality parameters measured, the elevated ammonia levels observed may indicate fresh human input of urine. Alternatively, a different chemical component of urine, such as urea, could be used to measure probable urine concentrations in the water.

In order to evaluate the resulting public health effect of this event, a pre- and post-jump health survey would allow participants and researchers to assess whether the health symptoms that may have been experienced by participants after the jump could be due to pathogens that were screened in this experiment. Additionally, more samples could be gathered in the weeks before and after the jump to see how the ecosystem of the lake recovers after a large human impact event. Moreover, source-tracking of indicator organisms could be done by collecting sediment samples and analyzing them to identify and quantify bacteria (Allen et al. 1953; Van Donsel and Geldreich 1971; LaLiberte and Grimes 1982; Doyle et al. 1992). As a result, the enumeration of these bacteria and viruses from sediment samples will more accurately reflect their presence in aquatic environments and epidemiological impact than enumeration from water samples.

Although Mirror Lake is an urban lake unique to the Ohio State campus, the conclusions on the potential public health impacts from this case study are significant. This study had one of the largest number of participants ever recorded for a single swimming event in a known volume of water. In addition to using previously accepted EPA chemical and microbiological standards to assess water quality, this study is one of the first to show that *Enterococci* levels associated with bather shedding may be indicative of human fecal contribution to the water column. This was observed when rising *E. coli* and *Enterococci* levels, both traditional indicator organisms, corresponded with increases in human fecal genetic markers. Although this experiment was conducted during a single unique sampling event, more trials of this event need to be conducted in order to further assess the strength of this observed association between *Enterococci* and human

fecal genetic markers. Further sampling could provide insight into using *Enterococci* as a predictor in assessing human fecal contribution to this event and other recreational bodies of water in the future.

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TABLES

Table 1: Summary of water quality parameters measured during the Mirror Lake Jump, 2010.

Time	No. of People in Water	Temp (°C)	pH	Conductivity (S/cm)	DO (mg/L)	Chlorine (mg/L)	Ammonia (mg/L)	Phosphorus (mg/L)	Chl A (ug/L)	Phycocyanin (ug/L)
6 PM	0	10.3	8.3	350	13.93	0.12	0.02	0.44	24.61	0.50
8 PM	2	10.1	8.2	353	14.53	0.10	0.04	0.40	26.54	0.51
9 PM	0	10.0	8.2	350	15.17	0.10	0.03	0.42	29.35	0.51
10 PM	65	10.0	8.1	346	15.69	0.10	0.06	0.44	26.01	0.52
11 PM	165	9.9	8.0	346	15.14	0.11	0.05	0.50	35.78	0.55
12 AM	575	9.7	8.0	347	NA*	0.22	0.18	0.61	56.79	0.63
1 AM	0	9.7	8.0	355	12.68	0.15	0.19	0.47	48.68	0.64
2 AM	0	9.7	7.9	353	12.2	0.22	0.09	0.49	66.70	0.61

Table 2: Estimated enterococci concentrations potentially from bather shedding using the estimates described in Smith and Dufour (1993) and Elmir et al. (2007).

Time	People	Total People Over Hour	Est Ent. CFU Shed/Bather (Smith and Dufour, 1993)	2 Min. Shed Est.	1 min. Shed Est.	Total Ent Over Hour	Approx Mirror Lake Volume Used (Liters)	Predicted Cum Est. Ent. Shed/L	Predicted Cum Est. Ent. Shed/100 ml	Observed Shedding (CFU/100 ml)
6:00 PM	0	0	66000	52800	26400	0	774000	0	0	36.33
8:00 PM	2	120	66000	52800	26400	3168000	774000	4.09	0.41	65
9:00 PM	0	0	66000	52800	26400	0	774000	0	0	88.67
10:00 PM	65	3900	66000	52800	26400	102960000	774000	133.02	13.30	59
11:00 PM	165	9900	66000	52800	26400	261360000	774000	337.67	33.77	60
12:00 AM	575	34500	66000	52800	26400	910800000	774000	1176.74	117.67	9400

Time	People	Total People Over Hour	Est Ent. CFU Shed/Bather (Elmir et al. 2007)	2 Min. Shed Est.	1 min. Shed Est.	Total Ent Over Hour	Approx Mirror Lake Volume Used	Predicted Cum Est. Ent. Shed/L	Predicted Cum Est. Ent. Shed/100 ml	Observed Shedding (CFU/100 ml)
6:00 PM	0	0	550000	440000	220000	0	774000	0	0	36.33
8:00 PM	2	120	550000	440000	220000	26400000	774000	34.11	3.41	65
9:00 PM	0	0	550000	440000	220000	0	774000	0	0	88.67
10:00 PM	65	3900	550000	440000	220000	858000000	774000	1108.53	110.85	59
11:00 PM	165	9900	550000	440000	220000	2178000000	774000	2813.95	281.40	60
12:00 AM	575	34500	550000	440000	220000	7590000000	774000	9806.20	980.62	9400

Total People Over Hour = (# of People Observed in Water for 1 min.) (60 min/1hr)

Estimated Enterococci CFU Shed/Bather = Counts from previous studies by Smith and Dufour (1993) and Elmir et al. (2007)
2 Minute Shed Estimate = Estimate based on observed assumption that most (~80 %) of Enterococci are shed from bathers within first 2 minutes of submersion

1 Minute Shed Estimate = Assumes that person will be in water for ~ 1 minute with given cold weather conditions (Exposure)

Total Enterococci Shed Predicted from All Swimmers Over 1 Hour = (1 Minute Shed Estimate) x (Total People Over Hour)

Approximate Mirror Lake Volume Used = 30% of Total Volume (Based on location of swimmers)

Predicted Cumulative Enterococci Shed Per Liter = Total Enterococci Shed / Approximate Mirror Lake Volume

Predicted Cumulative Enterococci Shed Per 100 mL = 0.1 (Predicted Cumulative Enterococci Shed Per Liter)

FIGURES

Figure 1: Overhead image of Mirror Lake. The majority of swimmers stayed near the eastern shore of the lake shaded by the dotted lines (~30% of the lake area), with the sampling location indicated.

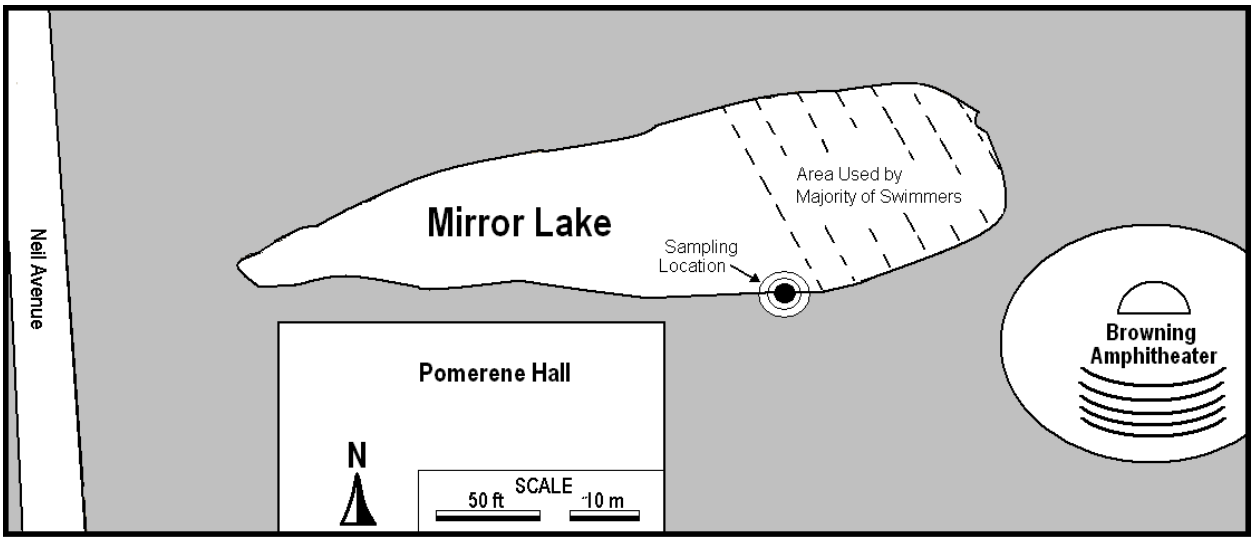


Figure 2: E. coli, enterococci, *and turbidity during the Mirror Lake Jump. The numbers above each time period indicate the number of people in the water at the time.* E. coli *and* enterococci *were enumerated using culture-based plate counts methods. The increase in turbidity, E. coli, and Enterococci all corresponded to the increase in the number of people.*

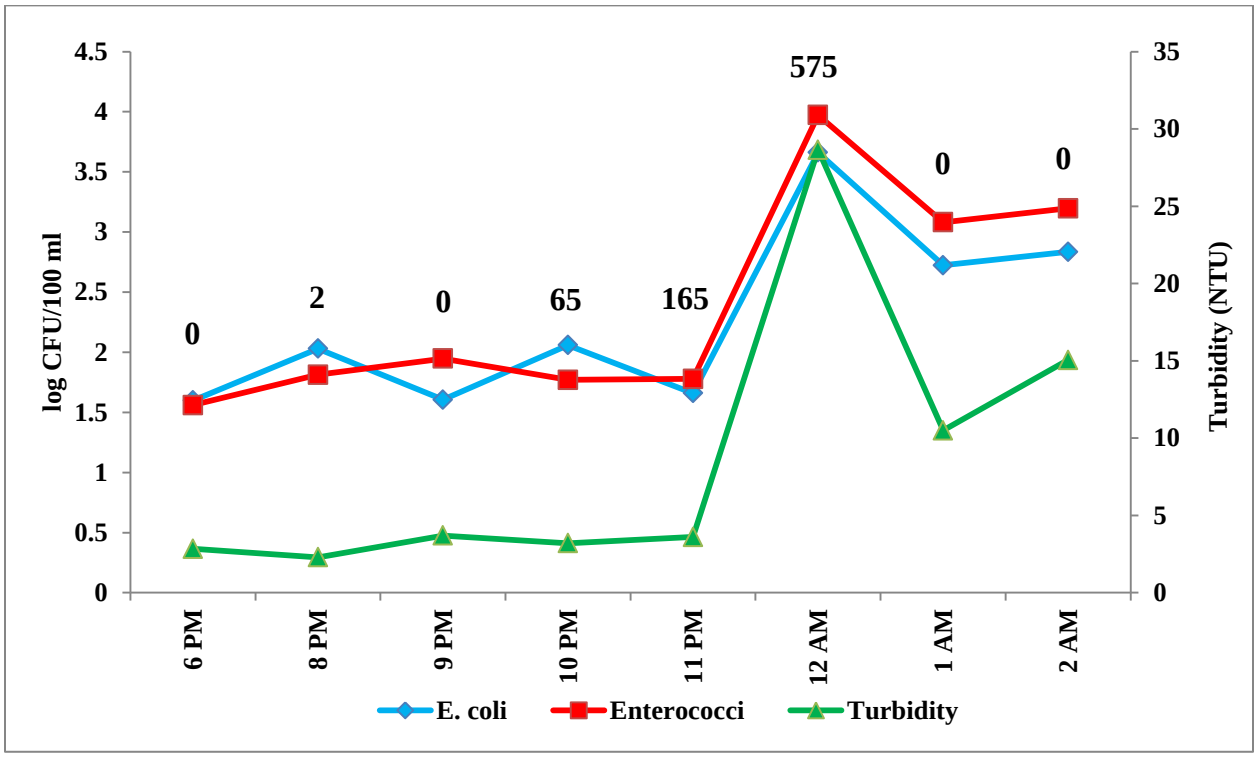
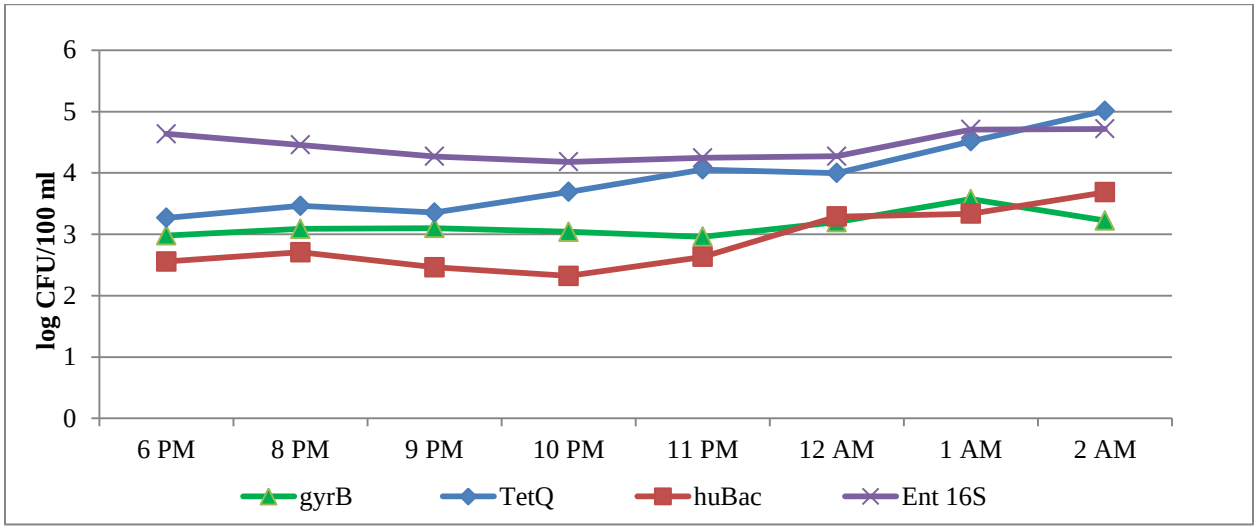


Figure 3: Overall fecal contamination measured by multiple genetic markers during the Mirror Lake Jump.



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